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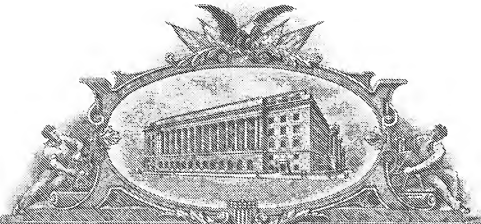

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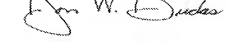

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV372403529US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

DICATIONIC COMPOUNDS FOR ACTIVITY AGAINST TRICHOMONAS VAGINALIS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

[X] Customer Number: 25297

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 64
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[ ] Other (specify)
[ ] Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[ ] Applicant claims small entity status. See 37 CFR 1.27.
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[ ] No.
[X] Yes, the name of the U.S. Government agency and the Government contract number are: NIH Grant No. NAID RO1AI46365

Respectfully submitted,

SIGNATURE

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Date 03/08/04

REGISTRATION NO. 39,395 *(If appropriate)*

Docket Number: 1523/2 PROV

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

JENKINS
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& TAYLOR

patent attorneys

March 8, 2004

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Re: U.S. Provisional Patent Application for
DICATIONIC COMPOUNDS FOR ACTIVITY AGAINST *TRICHOMONAS VAGINALIS*
Our Ref. No. 1523/2 PROV

Sir:

Please find enclosed the following:

1. Provisional Application Cover Sheet (1 pg.) in duplicate;
2. U.S. Provisional Patent Application (64 pgs.);
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Please contact our offices if there are any questions.

The Commissioner is hereby authorized to charge any fees associated with the filing of this correspondence to Deposit Account Number **50-0426**.

Respectfully submitted,

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UNITED STATES PROVISIONAL PATENT APPLICATION

DICATIONIC COMPOUNDS FOR ACTIVITY AGAINST *TRICHOMONAS VAGINALIS*

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Patty Wilson

Description

DICATIONIC COMPOUNDS FOR ACTIVITY AGAINST *TRICHOMONAS VAGINALIS*

Government Interest

The work was funded by the Office of Minority and Women's Health, National Center for Infectious Diseases, Centers for Disease Control and Prevention. The compound synthesis activities were supported by NIH grant No. NAID RO1AI46365.

Technical Field

The presently disclosed subject matter relates to methods of treating trichomoniasis infections with novel dicationic compounds, processes of synthesizing novel dicationic compounds, and to the novel compounds themselves. More particularly, the presently disclosed subject matter relates to methods of treating infections caused by the protozoan parasite *Trichomonas vaginalis* with novel dicationic compounds.

Abbreviations

δ	=	chemical shift
Ac	=	acetyl
AcO	=	acetoxyl
AcOH	=	acetic acid
Ac_2O	=	acetic anhydride
Am	=	amidine
AmOH	=	amidoxime
Bn	=	benzyl
Bu	=	butyl

	Bz	=	benzoyl
	°C	=	degrees Celsius
	calcd	=	calculated
	CDC	=	Centers for Disease Control
5	$CDCl_3$	=	deuterated chloroform
	cm	=	centimeters
	dec	=	decomposition point
	DIBAL	=	diisobutylaluminium hydride
	DMF	=	dimethylformamide
10	DMSO	=	dimethylsulfoxide
	D_2O	=	deuterium oxide
	Et	=	ethyl
	Et_2O	=	diethyl ether
	EtOAc	=	ethyl acetate
15	EtOH	=	ethanol
	g	=	grams
	GC	=	gas chromatography
	GLC	=	gas-liquid chromatography
	h	=	hours
20	HCl	=	hydrogen chloride
	HPLC	=	high-pressure liquid chromatography
	Hz	=	hertz
	ip	=	intraperitoneal
	IR	=	infrared
25	kg	=	kilograms
	KO-t-Bu	=	potassium *tert*-butoxide
	M	=	molar
	Me	=	methyl
	MeO	=	methoxyl
30	MHz	=	megahertz
	mL	=	milliliters
	MLC	=	minimal lethal concentration
	mm	=	millimeters

	mmol	=	millimole
	μM	=	micromolar
	m.p.	=	melting point
	MS	=	mass spectroscopy
	NaCl	=	sodium chloride
	$NaHCO_3$	=	sodium bicarbonate
	Na_2CO_3	=	sodium carbonate
	Na_2HPO_4	=	sodium hydrogen phosphate
	Na_2SO_4	=	sodium sulfate
	NaOH	=	sodium hydroxide
	NBS	=	*N*-bromosuccinimide
	NH_2OH•HCl	=	hydroxylamine hydrochloride
	NMR	=	nuclear magnetic resonance
	p	=	para
	Ph	=	phenyl
	Pd-C	=	10% palladium on carbon
	psi	=	pounds per square inch
	po	=	oral
	spp.	=	species
	TBME	=	tert-butyldimethyl ether
	THF	=	tetrahydrofuran
	TLC	=	thin-layer chromatography
	TMS	=	trimethylsilyl
	T. vaginalis	=	*Trichomonas vaginalis*
	TYM	=	trypticase-yeast-maltose medium
	UV	=	ultraviolet

Background Art

Trichomoniasis is a common sexually transmitted disease caused by the protozoan parasite *Trichomonas vaginalis*. An estimated 170 million persons are infected with *T. vaginalis* worldwide. See World Health Organization, An Overview of Selected Curable Sexually Transmitted Diseases, *in* Global Program on AIDS (World Health Organization, Geneva,

Switzerland), 2-27 (1995). Clinical manifestations range from an asymptomatic presentation to vaginitis, dyspareunia, and strawberry cervix in women and urethritis in men. In addition to these direct symptoms, trichomoniasis has also been associated with premature birth, low infant birth weight, and increased susceptibility to HIV infection. See Cotch, M. F., et al., *Sex. Transm. Dis.*, 24, 353-360 (1997); Sorvillo, F., et al., *Lancet*, 351, 213-214 (1998).

Metronidazole has been the principal drug prescribed for treatment of trichomoniasis infections since it was introduced in 1960. Durel, P., et al., *Br. J. Vener. Dis.* 36, 21-26 (1960). Although resistance to metronidazole was first reported in 1962, see Robinson, S. C., *Can. Med. Assoc. J.*, 86, 665 (1962), it is still effective, successfully treating approximately 90-95% of infections. See Centers for Disease Control and Prevention, Sexually Transmitted Diseases Treatment Guidelines. MMWR: Morbidity and Mortality Weekly Report., 42(RR-14), 70-72 (1993). Metronidazole treatment does not cure all patients, however, and recognition of resistance is increasing. Requests to the Centers for Disease Control (CDC) for evaluation of metronidazole resistance in clinical isolates have increased from fifteen in 1995 to over 100 in 2003. In addition, side effects such as gastrointestinal discomfort and nausea are commonly reported. See Smilack, J. D., et al., *Mayo Clin. Proc.*, 66, 1270-1280 (1991). Along with hypersensitivity reactions, these side effects can be severe enough to preclude metronidazole use for treating some individuals. See Kurohara, M. L., et al., *J. Allergy Clin. Immunol.*, 88, 279-280 (1991).

The efficacy of tinidazole against *T. vaginalis* isolates at lower minimal lethal concentrations (MLCs) than metronidazole has been reported. See Crowell, A. L., et al., *Antimicrob. Agents Chemother.*, 47, 1407-1409 (2003). This finding also is supported by clinical observations. See Sobel, J. D., et al., *Clin. Infect. Dis.*, 33, 1341-1346 (2001). Like metronidazole, tinidazole is a 5-nitroimidazole and isolates with very high levels of resistance to metronidazole also have increased tinidazole MLCs. See Crowell, A. L., et al., *Antimicrob. Agents Chemother.*, 47, 1407-1409 (2003).

In addition, although tinidazole use results in fewer common side effects than metronidazole, it is possible that persons with hypersensitivity reactions to metronidazole may also have adverse reactions to tinidazole. Taken together, although tinidazole may prove to be useful in many cases of metronidazole treatment failure, identification of non-nitroimidazole compounds that have efficacy against trichomonads is desirable.

Dicationic, aromatic diamidine compounds that bind the minor groove of DNA have antimicrobial activity against a wide spectrum of protozoan parasites. See Tidwell, R. R. and D. W. Boykin, Dicationic DNA Minor Groove Binders as Antimicrobial Agents, *in* Small Molecule DNA and RNA Binders: From Synthesis to Nucleic Acid Complexes, vol. 2. (M. Demeunynck, C. Bailly, and W. D. Wilson, ed., Wiley-VCH, New York, 2003), 416-460. For example, pentamidine is used to treat African trypanosomiasis and antimony-resistant leishmaniasis. Pentamidine, however, must be administered parenterally, it causes potentially severe host side effects, and drug resistance among parasites is emerging. These factors have led to recent research on compounds that are structurally related to pentamidine and retain anti-parasite activity, but demonstrate decreased toxicity for mammalian cells. Chemically synthesized diamidine compounds that preferentially bind the minor groove of DNA have activity against *Cryptosporidium parvum*, see Blagburn, B. L., et al., *Antimicrob. Agents and Chemother.*, 42, 2877-2882 (1998), *Leishmania donovani*, see Bell, C. A., et al., *Antimicrob. Agents and Chemother.*, 34, 1381-1386 (1991); Stephens, C. E., et al., *Bioorganic & Medicinal Chem. Lett.*, 13, 2065-2069 (2003), *Plasmodium falciparum*, see Bell, C. A., et al., *Antimicrob. Agents and Chemother.*, 34, 1381-1386 (1991), *Trypanosoma brucei*, see Ismail, M., et al., *J. Med. Chem.*, 46, 4761-4769 (2003), *T. cruzi*, see Stephens, C. E., et al., *Bioorganic & Medicinal Chem. Lett.*, 13, 2065-2069 (2003), and the fungi *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, see Del Poeta, M., et al., *Antimicrob. Agents and Chemother.*, 42, 2495-2510 (1998); Del Poeta, M., et al, *Antimicrob. Agents and Chemother.*, 42, 2503-2502 (1998). In addition, appropriate chemical design of prodrugs for these compounds can confer systemic bioavailability following oral administration. See Tidwell, R. R. and

D. W. Boykin, Dicationic DNA Minor Groove Binders as Antimicrobial Agents, *in* Small Molecule DNA and RNA Binders: From Synthesis to Nucleic Acid Complexes, vol. 2. (M. Demeunynck, C. Bailly, and W. D. Wilson, ed., Wiley-VCH, New York, 2003), 416-460.

Summary

It is an object of the presently disclosed subject matter to provide methods for treating infections caused by the protozoan parasite *Trichomonas vaginalis* in a subject in need thereof. It is another object of the presently disclosed subject matter to provide compounds that are useful in the treatment of *Trichomonas vaginalis* infections. It is another object of the presently disclosed subject matter to provide pharmaceutical formulations for use in the treatment of *Trichomonas vaginalis* infections. It is another object of the presently disclosed subject matter to provide a process of synthesizing compounds that are useful in the treatment of *Trichomonas vaginalis* infections.

Certain objects of the presently disclosed subject matter having been stated hereinabove, which are addressed in whole or in part by the presently disclosed subject matter, other aspects and objects will become evident as the description proceeds when taken in connection with the accompanying Examples as best described herein below.

Detailed Description

The presently disclosed subject matter will now be described more fully hereinafter with reference to the accompanying Examples, in which preferred embodiments are shown. The presently disclosed subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the embodiments to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs. All

publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. Throughout the specification and claims, a given chemical formula or name shall encompass all optical and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

I. Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Throughout the specification and claims, a given chemical formula or name shall encompass all optical and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

As used herein the term "alkyl" refers to C_{1-20} inclusive, linear (*i.e.*, "straight-chain"), branched, or cyclic, saturated or at least partially and in some cases fully unsaturated (*i.e.*, alkenyl and alkynyl) hydrocarbon chains, including for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, pentyl, hexyl, octyl, ethenyl, propenyl, butenyl, pentenyl, hexenyl, octenyl, butadienyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, and allenyl groups. "Branched" refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain. "Lower alkyl" refers to an alkyl group having 1 to about 8 carbon atoms (*i.e.*, a C_{1-8} alkyl), e.g., 1, 2, 3, 4, 5, 6, 7, or 8 carbon atoms. "Higher alkyl" refers to an alkyl group having about 10 to about 20 carbon atoms, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. In certain embodiments, "alkyl" refers, in particular, to C_{1-8} straight-chain alkyls. In other embodiments, "alkyl" refers, in particular, to C_{1-8} branched-chain alkyls.

Alkyl groups can optionally be substituted with one or more alkyl group substituents, which can be the same or different. The term "alkyl group substituent" includes but is not limited to alkyl, halo, arylamino, acyl, hydroxyl, aryloxyl, alkoxyl, alkylthio, arylthio, aralkyloxyl, aralkylthio, carboxyl, alkoxycarbonyl, oxo, and cycloalkyl. There can be optionally inserted along

the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl (also referred to herein as "alkylaminoalkyl"), or aryl.

Alkyl groups can further be joined to form a cycloalkyl group or a cycloheteroalkyl group. "Cyclic" and "cycloalkyl" refer to a non-aromatic mono- or multicyclic ring system of about 3 to about 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The cycloalkyl group can be optionally partially unsaturated. The cycloalkyl group also can be optionally substituted with an alkyl group substituent as defined herein, oxo, and/or alkylene. There can be optionally inserted along the cyclic alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl, or aryl, thus providing a cycloheteroalkyl group. Representative monocyclic cycloalkyl rings include cyclopentyl, cyclohexyl, and cycloheptyl. Multicyclic cycloalkyl rings include adamantyl, octahydronaphthyl, decalin, camphor, camphane, and noradamantyl. Representative cycloheteroalkyl groups include piperidine and morpholine.

The term "aryl" is used herein to refer to an aromatic substituent that can be a single aromatic ring, or multiple aromatic rings that are fused together, linked covalently, or linked to a common group, such as, but not limited to, a methylene or ethylene moiety. The common linking group also can be a carbonyl, as in benzophenone, or oxygen, as in diphenylether, or nitrogen, as in diphenylamine. The term "aryl" specifically encompasses heterocyclic aromatic compounds. The aromatic ring(s) can comprise phenyl, naphthyl, biphenyl, diphenylether, diphenylamine and benzophenone, among others. In particular embodiments, the term "aryl" means a cyclic aromatic comprising about 5 to about 10 carbon atoms, e.g., 5, 6, 7, 8, 9, or 10 carbon atoms, and including 5- and 6-membered hydrocarbon and heterocyclic aromatic rings.

The aryl group can be optionally substituted with one or more aryl group substituents, which can be the same or different, wherein "aryl group substituent" includes alkyl, aryl, aralkyl, hydroxyl, alkoxyl, aryloxyl, aralkyloxyl, carboxyl, acyl, halo, nitro, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, acyloxyl, acylamino, aroylamino, carbamoyl, alkylcarbamoyl,

dialkylcarbamoyl, arylthio, alkylthio, alkylene, and –NR'R", wherein R' and R" can each be independently hydrogen, alkyl, aryl, and aralkyl.

Specific examples of aryl groups include, but are not limited to, cyclopentadienyl, phenyl, furan, thiophene, pyrrole, pyran, pyridine, imidazole, benzimidazole, isothiazole, isoxazole, pyrazole, pyrazine, triazine, pyrimidine, quinoline, isoquinoline, indole, carbazole, and the like.

As used herein, the terms "substituted alkyl" and "substituted aryl" include alkyl and aryl groups, as defined herein, in which one or more atoms or functional groups of the aryl or alkyl group are replaced with another atom or functional group, including for example, halogen, aryl, alkyl, alkoxyl, hydroxyl, nitro, amino, alkylamino, dialkylamino, sulfate, and mercapto.

"Alkylene" refers to a straight or branched bivalent aliphatic hydrocarbon group having from 1 to about 20 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. The alkylene group can be straight, branched or cyclic. The alkylene group can be also optionally unsaturated and/or substituted with one or more "alkyl group substituents." There can be optionally inserted along the alkylene group one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms (also referred to herein as "alkylaminoalkyl"), wherein the nitrogen substituent is alkyl as previously described. Exemplary alkylene groups include, but are not limited to, methylene ($–CH_2–$); ethylene ($–CH_2–CH_2–$); propylene ($–(CH_2)_3–$); cyclohexylene ($–C_6H_{10}–$); –CH=CH—CH=CH–; –CH=CH–CH_2–; $–(CH_2)_q–N(R)–(CH_2)_r–$, wherein each of q and r is independently an integer from 0 to about 20, e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, and R is hydrogen or lower alkyl; methylenedioxyl ($–O–CH_2–O–$); and ethylenedioxyl ($–O–(CH_2)_2–O–$). An alkylene group can have about 2 to about 3 carbon atoms and can further have 6-20 carbons.

As used herein, the term "acyl" refers to an organic acid group wherein the -OH of the carboxyl group has been replaced with another substituent (i.e., as represented by RCO—, wherein R is an alkyl or an aryl group as defined herein). As such, the term "acyl" specifically includes arylacyl groups, such as an acetylfuran and a phenacyl group. Specific examples of acyl groups include acetyl and benzoyl.

"Alkoxyl" or "alkoxyalkyl" refer to an alkyl–O– group wherein alkyl is as previously described. The term "alkoxyl" as used herein can refer to $C_{1\text{-}20}$ inclusive, linear, branched, or cyclic, saturated or unsaturated oxo-hydrocarbon chains, including, for example, methoxyl, ethoxyl, propoxyl, isopropoxyl, butoxyl, t-butoxyl, and pentoxyl.

"Aryloxyl" refers to an aryl–O– group wherein the aryl group is as previously described. The term "aryloxyl" as used herein can refer to phenyloxyl or hexyloxyl, and alkyl, halo, or alkoxyl substituted phenyloxyl or hexyloxyl.

"Aralkyl" refers to an aryl–alkyl– group wherein aryl and alkyl are as previously described. Exemplary aralkyl groups include benzyl, phenylethyl, and naphthylmethyl.

"Aralkyloxyl" refers to an aralkyl–O– group wherein the aralkyl group is as previously described. An exemplary aralkyloxyl group is benzyloxyl.

"Dialkylamino" refers to an –NRR' group wherein each of R and R' is independently an alkyl group as previously described. Exemplary alkylamino groups include ethylmethylamino, dimethylamino, and diethylamino.

"Alkoxycarbonyl" refers to an alkyl–O–CO– group. Exemplary alkoxycarbonyl groups include methoxycarbonyl, ethoxycarbonyl, butyloxycarbonyl, and t-butyloxycarbonyl.

"Aryloxycarbonyl" refers to an aryl–O–CO—group. Exemplary aryloxycarbonyl groups include phenoxy- and naphthoxy-carbonyl.

"Aralkoxycarbonyl" refers to an aralkyl–O–CO– group. An exemplary aralkoxycarbonyl group is benzyloxycarbonyl.

"Carbamoyl" refers to an H_2N–CO– group.

"Alkylcarbamoyl" refers to a R'RN–CO– group wherein one of R and R' is hydrogen and the other of R and R' is alkyl as previously described.

"Dialkylcarbamoyl" refers to a R'RN–CO– group wherein each of R and R' is independently alkyl as previously described.

"Acyloxyl" refers to an acyl–O– group wherein acyl is as previously described.

"Acylamino" refers to an acyl–NH– group wherein acyl is as previously described.

"Aroylamino" refers to an aroyl–NH– group wherein aroyl is as previously described.

The term "amino" refers to the $–NH_2$ group.

The term "carbonyl" refers to the –(C=O)– group.

The term "carboxyl" refers to the –COOH group.

The terms "halo", "halide", or "halogen" as used herein refer to fluoro, chloro, bromo, and iodo groups.

The term "hydroxyl" refers to the –OH group.

The term "hydroxyalkyl" refers to an alkyl group substituted with an –OH group.

The term "mercapto" refers to the –SH group.

The term "oxo" refers to a compound described previously herein wherein a carbon atom is replaced by an oxygen atom.

The term "nitro" refers to the $–NO_2$ group.

The term "thio" refers to a compound described previously herein wherein a carbon or oxygen atom is replaced by a sulfur atom.

The term "sulfate" refers to the $–SO_4$ group.

The term "metal alkyl" refers to a compound of the general formula MR_n, wherein M is a metal atom, including, but not limited to aluminum, boron, magnesium, zinc, gallium, indium, antimony and related metals, R is an alkyl group as defined herein, and n is an integer.

When the term "independently selected" is used, the substituents being referred to (e.g., R groups, such as groups R_1 and R_2, or groups X and Y), can be identical or different. For example, both R_1 and R_2 can be substituted alkyls, or R_1 can be hydrogen and R_2 can be a substituted alkyl, etc.

A named "R", "R'," "X," "Y," "Y'", "A," "A'", "B," "L," or "Z" group will generally have the structure that is recognized in the art as corresponding to a group having that name, unless specified otherwise herein. For the purposes of illustration, certain representative "R," "X," "Y", and "A" groups as set forth above are defined below. These definitions are intended to supplement and illustrate, not preclude, the definitions that would be apparent to one of ordinary skill in the art upon review of the present disclosure.

The term "aprotic solvent" refers to a solvent molecule which can neither accept nor donate a proton. Typical aprotic solvents include, but are not limited to, acetone, acetonitrile, benzene, butanone, butyronitrile, carbon tetrachloride, chlorobenzene, chloroform, 1,2-dichloroethane, dichloromethane, diethyl ether, dimethylacetamide, *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), 1,4-dioxane, ethyl acetate, ethylene glycol dimethyl ether, hexane, *N*-methylpyrrolidone, pyridine, tetrahydrofuran (THF), and toluene. Certain aprotic solvents are polar solvents. Examples of polar aprotic solvents include, but are not limited to, acetone, acetonitrile, butanone, *N*,*N*-dimethylformamide, and dimethylsulfoxide. Certain aprotic solvents are non-polar solvents. Examples of nonpolar, aprotic solvents include, but are not limited to, diethyl ether, aliphatic hydrocarbons, such as hexane, aromatic hydrocarbons, such as benzene and toluene, and symmetrical halogenated hydrocarbons, such as carbon tetrachloride.

The term "protic solvent" refers to a solvent molecule which contains a hydrogen atom bonded to an electronegative atom, such as an oxygen atom or a nitrogen atom. Typical protic solvents include, but are not limited to, carboxylic acids, such as acetic acid, alcohols, such as methanol and ethanol, amines, amides, and water.

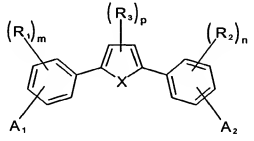
The term "reflux" and grammatical derivations thereof refer to boiling a liquid, such as a solvent, in a container, such as a reaction flask, with which a condenser is associated, thereby facilitating continuous boiling without loss of liquid, due to the condensation of vapors on the interior walls of the condenser.

II. Methods of Treating Trichomoniasis Infections

Subjects with trichomoniasis infections can be treated by methods described herein. These infections can be caused by the protozoan parasite *Trichomonas vaginalis*. The methods of the presently disclosed subject matter are useful for treating these conditions in that they inhibit the onset, growth, or spread of the condition, cause regression of the condition, cure the condition, or otherwise improve the general well-being of a subject afflicted with, or at risk of, contracting the condition.

The methods of treating a trichomoniasis infection comprise administering to a subject in need of treatment thereof an active compound as described herein. These active compounds, as set forth above, include compounds of Formula (I-IV), their corresponding prodrugs, and pharmaceutically acceptable salts of the compounds and prodrugs.

With regard to the presently described method embodiments, compounds of Formula (I) are defined as having a structure as follows:



(I)

wherein:

m is an integer from 0 to 4;

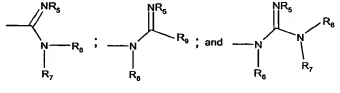
n is an integer from 0 to 4;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl,

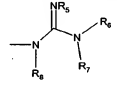
alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

In some embodiments, X is oxygen. In some embodiments, A_1 and A_2 comprise:

wherein R_6 and R_7 are H, and R_5 is selected from the group consisting of H, alkyl, and alkoxyl. In some embodiments, R_5 comprises H. In some embodiments, R_5 comprises an alkyl radical. In some embodiments, R_5 comprises an alkoxyl radical.

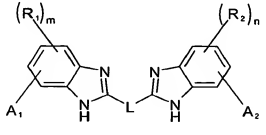
In some embodiments, A_1 and A_2 comprise:



wherein R_5, R_6, R_7, and R_8 are each H.

In some embodiments, the compound of Formula (I) is selected from the group consisting of: DB75; DB289; DB181; DB249; and DB673 as depicted in Scheme 1.

With regard to the presently described method embodiments, compounds of Formula (II) are defined as having a structure as follows:



(II)

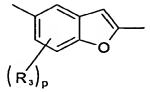
wherein:

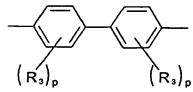
m is an integer from 0 to 3;

n is an integer from 0 to 3;

R_1 and R_2 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

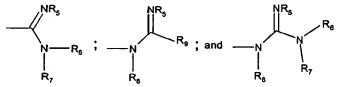
L is selected from the group consisting of:





wherein each p is independently an integer from 0 to 4 and each R_3 is independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

A_1 and A_2 are each independently selected from the group consisting of:

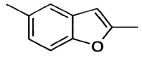


wherein:

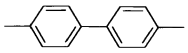
R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

In some embodiments, L comprises:



In some embodiments, L comprises:

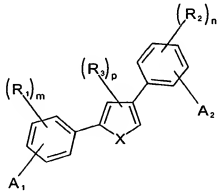


In some embodiments, A_1 and A_2 comprise

wherein R_6 and R_7 are H, and R_5 is selected from the group consisting of H and alkyl. In some embodiments, R_5 comprises hydrogen. In some embodiments, R_5 comprises an alkyl radical.

In some embodiments, the compound of Formula (II) is selected from the group consisting of: 4,4'-Bis{2-[(4-amidino) benzimidazolyl]}biphenyl tetrahydrochloride (DB507); and 2,5-Bis{2-[5-(*N*-isopropylamidino)benzimidazoyl]}benzo[b]furan tetrahydrochloride (DB364) as depicted in Scheme 1.

With regard to the presently described method embodiments, compounds of Formula (III) are defined as having a structure as follows:



(III)

wherein:

m is an integer from 0 to 4;

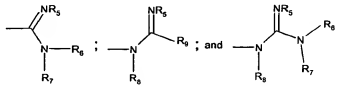
n is an integer from 0 to 4;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

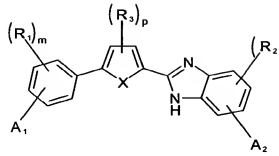
In some embodiments, X is oxygen.

In some embodiments, A_1 and A_2 comprise:

wherein R_5 , R_6 and R_7 are each H.

In some embodiments, the compound of Formula (III) is compound DB690 as depicted in Scheme 1.

With regard to the presently described method embodiments, compounds of Formula (IV) are defined as having a structure as follows:



(IV)

wherein:

m is an integer from 0 to 4;

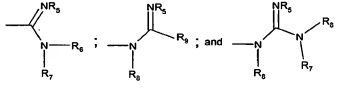
n is an integer from 0 to 3;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

In some embodiments, X is sulfur.

In some embodiments, A_1 and A_2 comprise

wherein R_5, R_6 and R_7 are each H.

In some embodiments, the compound of Formula (IV) is selected from compound DB818 as depicted in Scheme 1.

In some embodiments, the trichomoniasis infection is caused by the protozoan parasite *Trichomonas vaginalis*.

In some embodiments, the compound of Formula (I-IV) is administered in the form of a pharmaceutically acceptable salt. In some embodiments, the pharmaceutically acceptable salt comprises a hydrochloride salt.

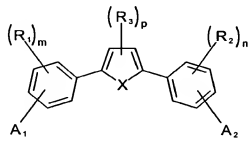
The subject treated in the presently disclosed subject matter in its many embodiments is desirably a human subject, although it is to be understood the methods described herein are effective with respect to all vertebrate species, which are intended to be included in the term "subject". The methods described herein are particularly useful in the treatment and/or prevention of infectious diseases in warm-blooded vertebrates. Thus, the methods can be used as treatment for mammals and birds.

More particularly, provided herein is the treatment of mammals, such as humans, as well as those mammals of importance due to being endangered (such as Siberian tigers), of economical importance (animals raised on farms for consumption by humans) and/or social importance (animals kept as pets or in zoos) to humans, for instance, carnivores other than humans (such as cats and dogs), swine (pigs, hogs, and wild boars), ruminants (such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels), and horses. Also provided herein is the treatment of birds, including the treatment of those kinds of birds that are endangered, kept in zoos, as well as fowl, and more particularly domesticated fowl, i.e., poultry, such as turkeys, chickens, ducks, geese, guinea fowl, and the like, as they also are of economical importance to humans. Thus, embodiments of the methods described herein include the treatment of livestock, including, but not limited to, domesticated swine (pigs and hogs), ruminants, horses, poultry, and the like.

III. Novel Compounds for Treating Trichomoniasis Infections

A. Novel Compounds of Formula (I-IV)

With regard to the presently described method embodiments, compounds of Formula (I) are defined as having a structure as follows:



(I)

wherein:

m is an integer from 0 to 4;

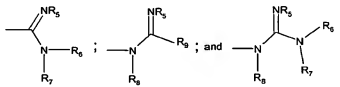
n is an integer from 0 to 4;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

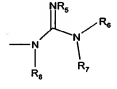
R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

In some embodiments, X is oxygen. In some embodiments, A_1 and A_2 comprise:

wherein R_6 and R_7 are H, and R_5 is selected from the group consisting of H, alkyl, and alkoxyl. In some embodiments, R_5 comprises H. In some embodiments, R_5 comprises an alkyl radical. In some embodiments, R_5 comprises an alkoxyl radical.

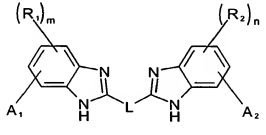
In some embodiments, A_1 and A_2 comprise:



wherein R_5, R_6, R_7, and R_8 are each H.

In some embodiments, the compound of Formula (I) is selected from the group consisting of: DB75; DB289; DB181; DB249; and DB673 as depicted in Scheme 1.

With regard to the presently described method embodiments, compounds of Formula (II) are defined as having a structure as follows:



(II)

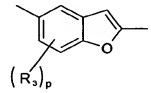
wherein:

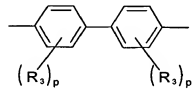
m is an integer from 0 to 3;

n is an integer from 0 to 3;

R_1 and R_2 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

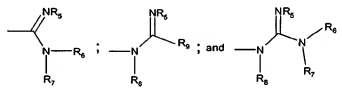
L is selected from the group consisting of:





wherein each p is independently an integer from 0 to 4 and each R_3 is independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

A_1 and A_2 are each independently selected from the group consisting of:

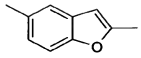


wherein:

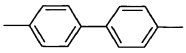
R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

In some embodiments, L comprises:



In some embodiments, L comprises:

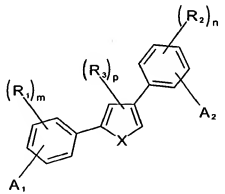


In some embodiments, A_1 and A_2 comprise

wherein R_6 and R_7 are H, and R_5 is selected from the group consisting of H and alkyl. In some embodiments, R_5 comprises hydrogen. In some embodiments, R_5 comprises an alkyl radical.

In some embodiments, the compound of Formula (II) is selected from the group consisting of: 4,4'-Bis{2-[(4-amidino) benzimidazolyl]}biphenyl tetrahydrochloride (DB507); and 2,5-Bis{2-[5-(*N*-isopropylamidino)benzimidazoyl]}benzo[b]furan tetrahydrochloride (DB364) as depicted in Scheme 1.

With regard to the presently described method embodiments, compounds of Formula (III) are defined as having a structure as follows:



(III)

wherein:

m is an integer from 0 to 4;

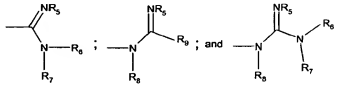
n is an integer from 0 to 4;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

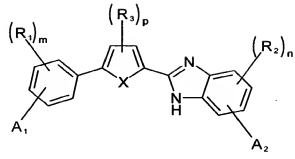
In some embodiments, X is oxygen.

In some embodiments, A_1 and A_2 comprise:

wherein R_5 , R_6 and R_7 are each H.

In some embodiments, the compound of Formula (III) is compound DB690 as depicted in Scheme 1.

With regard to the presently described method embodiments, compounds of Formula (IV) are defined as having a structure as follows:



(IV)

wherein:

m is an integer from 0 to 4;

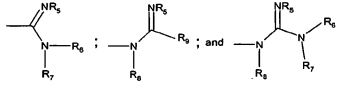
n is an integer from 0 to 3;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

B. Prodrugs

In representative embodiments, compounds disclosed herein are prodrugs. A prodrug means a compound that, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of the presently disclosed subject matter or an inhibitorily active metabolite or residue thereof. Prodrugs can increase the bioavailability of the compounds of the presently disclosed subject matter when such compounds are administered to a subject (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or can enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to a metabolite species, for example. A number of the compounds discussed in the Examples are prodrugs.

C. Pharmaceutically Acceptable Salts

Additionally, the active compounds can be administered as pharmaceutically acceptable salts. Such salts include the gluconate, lactate, acetate, tartarate, citrate, phosphate, borate, nitrate, sulfate, and hydrochloride salts. The salts of the compounds described herein can be prepared, in general, by reacting two equivalents of the base compound with the desired acid, in solution. After the reaction is complete, the salts are crystallized from solution by the addition of an appropriate amount of solvent in which the salt is insoluble. In some embodiments, the pharmaceutically acceptable salt is a hydrochloride salt. In other embodiments, the pharmaceutically acceptable salt is an acetate salt.

IV. Pharmaceutical Formulations

The compounds of Formula (I-IV), the pharmaceutically acceptable salts thereof, prodrugs corresponding to compounds of Formula (I-IV), and the pharmaceutically acceptable salts thereof, are all referred to herein as "active compounds." Pharmaceutical formulations comprising the aforementioned active compounds also are provided herein. These pharmaceutical formulations comprise active compounds as described herein, in a pharmaceutically acceptable carrier. Pharmaceutical formulations can be prepared for oral, intravenous, or aerosol administration as discussed in greater detail below. Also, the presently disclosed subject matter provides

such active compounds that have been lyophilized and that can be reconstituted to form pharmaceutically acceptable formulations for administration, as by intravenous or intramuscular injection.

The therapeutically effective dosage of any specific active compound, the use of which is in the scope of embodiments described herein, will vary somewhat from compound to compound, and patient to patient, and will depend upon the condition of the patient and the route of delivery. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with all weights being calculated based upon the weight of the active compound, including the cases where a salt is employed. Toxicity concerns at the higher level can restrict intravenous dosages to a lower level, such as up to about 10 mg/kg, with all weights being calculated based on the weight of the active base, including the cases where a salt is employed. A dosage from about 10 mg/kg to about 50 mg/kg can be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg can be employed for intramuscular injection. Preferred dosages are 1 μmol/kg to 50 μmol/kg, and more preferably 22 μmol/kg and 33 μmol/kg of the compound for intravenous or oral administration. The duration of the treatment is usually once per day for a period of two to three weeks or until the condition is essentially controlled. Lower doses given less frequently can be used prophylactically to prevent or reduce the incidence of recurrence of the infection.

In accordance with the present methods, pharmaceutically active compounds as described herein can be administered orally as a solid or as a liquid, or can be administered intramuscularly or intravenously as a solution, suspension, or emulsion. Alternatively, the compounds or salts also can be administered by inhalation, intravenously, or intramuscularly as a liposomal suspension. When administered through inhalation the active compound or salt should be in the form of a plurality of solid particles or droplets having a particle size from about 0.5 to about 5 microns, and preferably from about 1 to about 2 microns.

Pharmaceutical formulations suitable for intravenous or intramuscular injection are further embodiments provided herein. The pharmaceutical formulations comprise a compound of Formula (I-IV) described herein, a prodrug as described herein, or a pharmaceutically acceptable salt thereof, in any pharmaceutically acceptable carrier. If a solution is desired, water is the carrier of choice with respect to water-soluble compounds or salts. With respect to the water-soluble compounds or salts, an organic vehicle, such as glycerol, propylene glycol, polyethylene glycol, or mixtures thereof, can be suitable. In the latter instance, the organic vehicle can contain a substantial amount of water. The solution in either instance can then be sterilized in a suitable manner known to those in the art, and typically by filtration through a 0.22-micron filter. Subsequent to sterilization, the solution can be dispensed into appropriate receptacles, such as depyrogenated glass vials. Of course, the dispensing is preferably done by an aseptic method. Sterilized closures can then be placed on the vials and, if desired, the vial contents can be lyophilized.

In addition to compounds of Formula (I-IV) or their salts or prodrugs, the pharmaceutical formulations can contain other additives, such as pH-adjusting additives. In particular, useful pH-adjusting agents include acids, such as hydrochloric acid, bases or buffers, such as sodium lactate, sodium acetate, sodium phosphate, sodium citrate, sodium borate, or sodium gluconate. Further, the formulations can contain anti-microbial preservatives. Useful anti-microbial preservatives include methylparaben, propylparaben, and benzyl alcohol. The anti-microbial preservative is typically employed when the formulation is placed in a vial designed for multi-dose use. The pharmaceutical formulations described herein can be lyophilized using techniques well known in the art.

In yet another embodiment of the subject matter described herein, there is provided an injectable, stable, sterile formulation comprising a compound of Formula (I-IV), or a salt thereof, in a unit dosage form in a sealed container. The compound or salt is provided in the form of a lyophilizate, which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid formulation suitable for

injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the compound salt. When the compound or salt is substantially water-insoluble, a sufficient amount of emulsifying agent, which is physiologically acceptable, can be employed in sufficient quantity to emulsify the compound or salt in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Other pharmaceutical formulations can be prepared from the water-insoluble compounds disclosed herein, or salts thereof, such as aqueous base emulsions. In such an instance, the formulation will contain a sufficient amount of pharmaceutically acceptable emulsifying agent to emulsify the desired amount of the compound or salt thereof. Particularly useful emulsifying agents include phosphatidyl cholines and lecithin.

Additional embodiments provided herein include liposomal formulations of the active compounds disclosed herein. The technology for forming liposomal suspensions is well known in the art. When the compound is an aqueous-soluble salt, using conventional liposome technology, the same can be incorporated into lipid vesicles. In such an instance, due to the water solubility of the active compound, the active compound will be substantially entrained within the hydrophilic center or core of the liposomes. The lipid layer employed can be of any conventional composition and can either contain cholesterol or can be cholesterol-free. When the active compound of interest is water-insoluble, again employing conventional liposome formation technology, the salt can be substantially entrained within the hydrophobic lipid bilayer that forms the structure of the liposome. In either instance, the liposomes that are produced can be reduced in size, as through the use of standard sonication and homogenization techniques.

The liposomal formulations containing the active compounds disclosed herein can be lyophilized to produce a lyophilizate, which can be reconstituted with a pharmaceutically acceptable carrier, such as water, to regenerate a liposomal suspension.

Pharmaceutical formulations also are provided which are suitable for administration as an aerosol by inhalation. These formulations comprise a solution or suspension of a desired compound described herein or a salt

thereof, or a plurality of solid particles of the compound or salt. The desired formulation can be placed in a small chamber and nebulized. Nebulization can be accomplished by compressed air or by ultrasonic energy to form a plurality of liquid droplets or solid particles comprising the compounds or salts. The liquid droplets or solid particles should have a particle size in the range of about 0.5 to about 10 microns, more preferably from about 0.5 to about 5 microns. The solid particles can be obtained by processing the solid compound or a salt thereof, in any appropriate manner known in the art, such as by micronization. Most preferably, the size of the solid particles or droplets will be from about 1 to about 2 microns. In this respect, commercial nebulizers are available to achieve this purpose. The compounds can be administered via an aerosol suspension of respirable particles in a manner set forth in U.S. Patent No. 5,628,984, the disclosure of which is incorporated herein by reference in its entirety.

When the pharmaceutical formulation suitable for administration as an aerosol is in the form of a liquid, the formulation will comprise a water-soluble active compound in a carrier that comprises water. A surfactant can be present, which lowers the surface tension of the formulation sufficiently to result in the formation of droplets within the desired size range when subjected to nebulization.

As indicated, both water-soluble and water-insoluble active compounds are provided. As used herein, the term "water-soluble" is meant to define any composition that is soluble in water in an amount of about 50 mg/mL, or greater. Also, as used herein, the term "water-insoluble" is meant to define any composition that has a solubility in water of less than about 20 mg/mL. In some embodiments, water-soluble compounds or salts can be desirable whereas in other embodiments water-insoluble compounds or salts likewise can be desirable.

Examples

The following Examples have been included to illustrate modes of the presently disclosed subject matter. Certain aspects of the following Examples are described in terms of techniques and procedures found or contemplated

to work well in the practice of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

Materials and Methods

Parasite Isolates and Compounds

CDC reference strains 085 and 520 that are metronidazole resistant and sensitive, respectively, were maintained at 37°C in Diamond's trypticase-yeast-maltose medium (TYM; pH 6.0). Metronidazole, tinidazole, pentamidine, and berenil were purchased from Sigma Chemical Co. (St. Louis, Missouri, United States of America). DB75, see Das, B. P. and D. W. Boykin, *J. Med. Chem.*, 20, 531-536 (1977); DB289, see Boykin, D. W., et al., *Bioorganic and Med. Chem. Lett.*, 6:3017-3020 (1996); DB181 and DB249, see Boykin, D. W., et al., *J. Med. Chem.*, 41, 124-129 (1998); DB690, see Francesconi, I., et al., *J. Med. Chem.*, 42, 2260-2265 (1999); DB673, see Stephens, C., et al., *J. Med. Chem.*, 44, 1741-1748 (2001); and DB818 (Lee, et al., manuscript in preparation) were synthesized as previously reported. Purity was determined by NMR and TLC. The syntheses of DB507 and DB364 are outlined below.

4,4'-Bis{2-[(4-amidino) benzimidazolyl]}biphenyl tetrahydrochloride (DB507). A mixture of 4,4'-diformyl-1,1'-biphenyl (0.21 g, 0.001 mole), 4-amidino-1, 2-phenylenediamine hydrochloride hemihydrate (0.39 g, 0.002 mole) and 1,4-benzoquinone (0.216 g, 0.002 mole) in ethanol was heated at reflux for 12 h. The solvent was reduced to one third, followed by dilution with ether and then filtration, yielding a dark solid. The solid was dissolved in large volume of hot ethanol and filtered; the solution was treated with 10 mL HCl gas saturated ethanol and stirred. The solvent was reduced to one third and diluted with ether. A dark hydrochloride salt precipitated, which was filtered, washed with ether, and dried in vacuum at 75 °C for 24 h to yield 0.43 g (66%); mp >300 °C dec.; ^{1}H-NMR (DMSO-d_6): 8.35(d, 4H, J =

7.6 Hz), 8.21(s, 2H), 8.02(d, 4H, J = 7.6 Hz), 7.85(d, 4H, J = 8.4 Hz), 7.50(d, 4H, J = 8.4 Hz); ^{13}C NMR (DMSO-d_6): 166.0, 153.2, 141.4, 137.5, 128.4, 127.8, 126.9, 123.4, 122.6, 116.2, 115.1; FAB MS: m/e 483(M^++1); Analysis calculated for $C_{29}H_{22}N_8.4HCl.1.5H_2O$: C, 53.41; H, 4.46: N, 17.09. Found: C, 52.97; H, 4.61; N, 17.17.

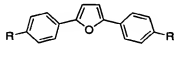
2,5-Bis{2-[5-(*N*-isopropylamidino)benzimidazoyl]}benzo[b]furan tetrahydrochloride (DB364). A protocol similar to that described above for DB507, involving the condensation of benzo[b]furan-2,5-dicarboxaldehyde and 4-*N*-isopropylamidino-1,2-phenylenediamine gave a metallic green solid in 69% yield; mp 285 °C-290 °C. ^1H NMR (DMSO-d_6/80°C) 8.71 (s, 1H), 8.36 (d, 1H, J = 8.8 Hz) 8.08(d, 2H, J = 9.2 Hz), 7.98 (d, 1H, J = 8.8 Hz), 7.96 (s, 1H), 7.85 (d, 1H, J = 8.8 Hz), 7.82 (d, 1H, J = 8.8 Hz), 7.64 (d, 1H, J = 8.8 Hz), 7.61 (d, 1H, J = 8.8 Hz), 4.02 (broad q, 2H, J = 6 Hz), 1.32(broad d, 12H, J = 6 Hz). ^{13}C NMR (DMSO-d_6/D_2O/80°C) 162.9, 162.6, 157.5, 152.9, 147.4, 145.3, 141.1, 138.7, 137.8, 134.9, 129.2, 126.9, 125.7, 125.0, 124.5, 123.8, 123.2, 121.4, 116.9, 116.1, 115.7, 115.5, 113.9, 109.4, 46.2, 46.1, 21.6 (signals overlap). Analysis calculated for $C_{30}H_{30}N_80$ 4HCl $0.5H_2O$: C, 53.49; H, 5.23; N, 16.64. Found: C, 53.53; H, 5.29; N, 16.45.

Assays

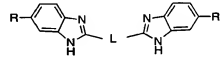
Compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma) and further diluted with Diamond's TYM media to reach assay concentrations. Two types of assays were performed on the synthesized cationic compounds being evaluated. An initial screen was performed using the standard MLC assay, see Crowell, A. L., et al., *Antimicrob. Agents Chemother.*, 47, 1407-1409 (2003); Meingassner, J. G. and J. Thurner, *Antimicrob. Agents Chemother.*, 15, 254-257 (1979), with a maximum concentration of 20μM for 44 test compounds. After 48 h of incubation at 37°C, plates were examined using an inverted phase-contrast microscope. The lowest drug concentration at which no motile trichomonads were observed was recorded as the MLC. Each compound was tested at least twice under both aerobic and anaerobic conditions. Anaerobic conditions were generated using a GasPak jar and CO_2-generating GasPak Plus anaerobic system envelopes (Becton Dickinson,

Sparks, Maryland, United States of America) and monitored with GasPak disposable anaerobic indicator strips (Becton Dickinson). Compounds that showed no activity were not tested further.

The structures of the six compounds that had some effect in the MLC assay as well as three related but ineffective compounds are shown in Scheme 1. To further evaluate their activity, a second type of assay was used to determine the concentration at which 50% of the parasite growth was inhibited (IC_{50}). In these assays, 0.5 μCi of tritiated thymidine (Perkin-Elmer, Boston, Massachusetts, United States of America) was added to each well of a standard assay at the initiation of culture. At 48 h of incubation under either aerobic or anaerobic conditions, cells were harvested onto glass fiber filters (Wallac, Turku, Finland) using a Tomtec cell harvester (Hamden, Connecticut, United States of America). Incorporated thymidine was detected using Betaplate (Wallac) scintillation fluid and plate reader. The resulting counts per minute over the compound's concentration range were utilized to calculate IC_{50}s using GraphPad Prism (GraphPad Software, Inc., San Diego, California, United States of America).

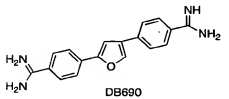


DB75 R = -C=NH(NH_2)
DB289 R = -C=NOMe(NH_2)
DB181 R = -C=N-*i*-Pr(NH_2)
DB249 R = -C=NH-*c*-cyclohexyl(NH_2)
DB673 R = -NH(C=NH)(NH_2)

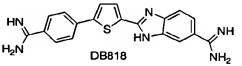


DB364 L =  R = -C=N-*i*-Pr(NH2)

DB507 L =  R = -C=NH(NH2)



DB690



DB818

Scheme 1. Structures of dicationic compounds evaluated in this study.

Results

Out of 44 dicationic compounds tested in the MLC assay, six showed sufficient activity for further evaluation and calculation of their IC_{50}. The structures of these compounds and three related compounds are shown in Scheme 1. Table 1 contains their *in vitro* IC_{50} activities against metronidazole-sensitive and -resistant *T. vaginalis* isolates as well as comparison data for the nitroimidazoles metronidazole and tinidazole. We have also included the evaluation of the classical antiprotozoan dicationic molecules pentamidine and berenil. Interestingly, both of these latter two compounds are not effective against *T. vaginalis*. In contrast, furamidine (DB75), the parent molecule in the 2,5-diphenylfuran family of diamidines, shows good activity that is comparable to that of metronidazole against 520, the metronidazole sensitive isolate. It is clearly more effective than either metronidazole or tinidazole against the resistant strain 085 under aerobic conditions. These data suggest, as expected based on structure, a different mode of action for DB75 as compared to the nitroimidazoles. The *N*-alkyl analogs of furamidine, DB181 and DB249, show similar *in vitro* effectiveness. Interestingly, DB673, a guanidino analog of DB75, and DB690, the 2,4-diphenylfuran isomer of DB75, are not effective against *T. vaginalis* in these assays. Thus, we find that the 2,5-diphenyl furan family of dications is quite effective versus *T. vaginalis in vitro*; but the activity is quite sensitive to structure as demonstrated by the lack of activity of DB690 and DB673.

DB289, the prodrug for DB75, was generally ineffective *in vitro* as the mammalian biochemical pathways necessary to convert it to the active form were not present. DB289, however, can be administered orally to provide systemic, efficacious concentrations of DB75 and is currently in phase II trials for treatment of African trypansomiasis, having successfully completed phase I clinical trials. See Tidwell, R. R., and D. W. Boykin, Dicationic DNA Minor Groove Binders as Antimicrobial Agents, *in* Small Molecule DNA and RNA Binders: From Synthesis to Nucleic Acid Complexes, vol. 2, (M. Demeunynck, C. Bailly, and W. D. Wilson, ed., Wiley-VCH, New York, 2003) p. 416-460.

The bis-benzimidazoles DB364 and DB507 are also effective anti-trichomonads. Interestingly, DB507 has activity against the metronidazole-

resistant 085 strain but not against the metronidazole-sensitive 520 isolate. Thus, DB507 may be a useful tool to evaluate the biochemical basis of *T. vaginalis* resistance to metronidazole. The most effective dication studied is the mono-benzimidazole DB818. This compound demonstrated IC_{50} values of 1 μMol or less for both metronidazole sensitive and resistant isolates under either aerobic or anaerobic conditions. The mono-benzimidazole DB818 has been successfully used *in vivo* to treat a different protozoal infection in an experimental model without overt evidence of toxicity to the host.

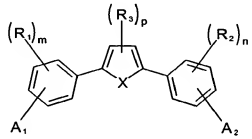
Table 1. IC_{50} values (μM ± SEM) of compounds evaluated in this study.

	085 aerobic		085 anaerobic		520 aerobic		520 anaerobic	
Compound	n		n		n		n	
Metronidazole	61	302.6 ± 22.2	62	12.3 ± 23.8	38	18.2 ± 4.25	31	1.89 ± 0.77
Tinidazole	5	45.1 ± 5.38	4	4.81 ± 1.17	2	1.48 ± 0.12	2	0.004 ± 0.0
Pentamidine	3	no effect	3	no effect	3	no effect	3	no effect
Berenil	3	no effect	3	no effect	3	no effect	3	no effect
DB75	8	8.12 ± 2.45	7	18.6 ± 19.8	4	18.6 ± 6.43	4	57.9 ± 19.6
DB289	3	39.1 ± 25.1	2	no effect	2	no effect	2	no effect
DB181	7	6.44 ± 3.89	7	9.41 ± 7.92	7	6.60 ± 1.76	7	3.91 ± 1.06
DB249	6	22.4 ± 13.7	6	10.2 ± 8.47	2	15.9 ± 0.74	3	13.9 ± 3.74
DB364	6	7.27 ± 3.76	5	37.6 ± 43.9	2	44.7 ± 9.33	3	143.1 ± 127
DB507	8	7.79 ± 4.20	6	25.3 ± 43.1	2	no effect	2	no effect
DB690	7	no effect	6	no effect	3	no effect	3	no effect
DB673	7	no effect	6	no effect	3	no effect	3	no effect
DB818	7	0.27 ± 0.04	7	0.50 ± 0.42	6	0.98 ± 0.19	6	1.24 ± 0.39

Claims

What is claimed is:

1. A method of treating a trichomoniasis infection in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (I):



(I)

wherein:

m is an integer from 0 to 4;

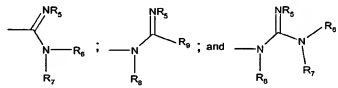
n is an integer from 0 to 4;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl,

alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

2. The method of Claim 1, wherein X is oxygen.

3. The method of Claim 1, wherein A_1 and A_2 comprise:

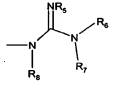
wherein R_6 and R_7 are H, and R_5 is selected from the group consisting of H, alkyl, and alkoxyl.

4. The method of Claim 3, wherein R_5 comprises H.

5. The method of Claim 3, wherein R_5 comprises an alkyl radical.

6. The method of Claim 3, wherein R_5 comprises an alkoxyl radical.

7. The method of Claim 1, wherein A_1 and A_2 comprise:



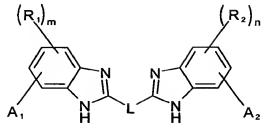
wherein R_5, R_6, R_7, and R_8 are each H.

8. The method of Claim 1, wherein the compound is selected from the group consisting of: DB75; DB289; DB181; DB249; and DB673 as depicted in Scheme 1.

9. The method of Claim 1, wherein the trichomoniasis infection is caused by the protozoan parasite *Trichomonas vaginalis*.

10. The method of Claim 1, wherein the compound of Formula I is administered in the form of a pharmaceutically acceptable salt.

11. A method of treating a trichomoniasis infection in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (II):



(II)

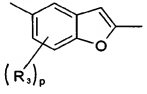
wherein:

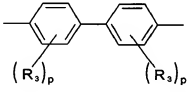
m is an integer from 0 to 3;

n is an integer from 0 to 3;

R_1 and R_2 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

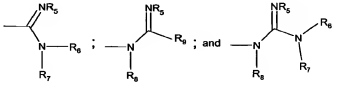
L is selected from the group consisting of:





wherein each p is independently an integer from 0 to 4 and each R_3 is independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

A_1 and A_2 are each independently selected from the group consisting of:

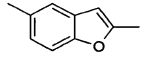


wherein:

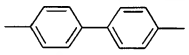
R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

12. The method of Claim 11, wherein L comprises:



13. The method of Claim 11, wherein L comprises:



14. The method of Claim 11, wherein A_1 and A_2 comprise

wherein R_6 and R_7 are H, and R_5 is selected from the group consisting of H and alkyl.

15. The method of Claim 14, wherein R_5 comprises hydrogen.

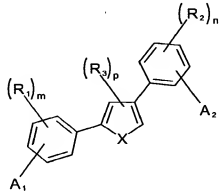
16. The method of Claim 14, wherein R_5 comprises an alkyl radical.

17. The method of Claim 11, wherein the compound is selected from the group consisting of: 4,4'-Bis{2-[(4-amidino) benzimidazolyl]}biphenyl tetrahydrochloride (DB507); and 2,5-Bis{2-[5-(*N*-isopropylamidino)benzimidazoyl]}benzo[b]furan tetrahydrochloride (DB364) as depicted in Scheme 1.

18. The method of Claim 11, wherein the trichomoniasis infection is caused by the protozoan parasite *Trichomonas vaginalis*.

19. The method of Claim 11, wherein the compound of Formula II is administered in the form of a pharmaceutically acceptable salt.

20. A method of treating a trichomoniasis infection in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (III):



(III)

wherein:

m is an integer from 0 to 4;

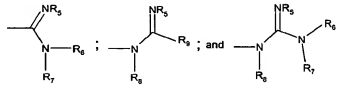
n is an integer from 0 to 4;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

21. The method of Claim 20, wherein X is oxygen.

22. The method of Claim 20, wherein A_1 and A_2 comprise:

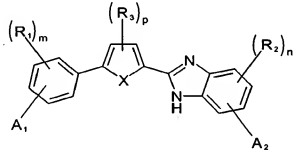
wherein R_5 , R_6 and R_7 are each H.

23. The method of Claim 20, wherein the compound is compound DB690 as depicted in Scheme 1.

24. The method of Claim 20, wherein the trichomoniasis infection is caused by the protozoan parasite *Trichomonas vaginalis.*

25. The method of Claim 20, wherein the compound of Formula III is administered in the form of a pharmaceutically acceptable salt.

26. A method of treating a trichomoniasis infection in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (IV):



(IV)

wherein:

m is an integer from 0 to 4;

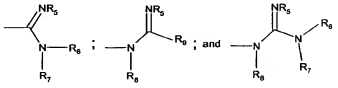
n is an integer from 0 to 3;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

27. The method of Claim 26, wherein X is sulfur.

28. The method of Claim 26, wherein A_1 and A_2 comprise

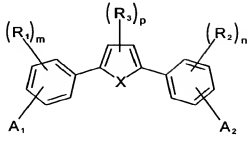
wherein R_5, R_6 and R_7 are each H.

29. The method of Claim 26, wherein the compound is selected from compound DB818 as depicted in Scheme 1.

30. The method of Claim 26, wherein the trichomoniasis infection is caused by the protozoan parasite *Trichomonas vaginalis*.

31. The method of Claim 26, wherein the compound of Formula IV is administered in the form of a pharmaceutically acceptable salt.

32. A compound of Formula (I):



(I)

wherein:

m is an integer from 0 to 4;

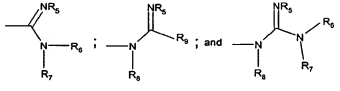
n is an integer from 0 to 4;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:

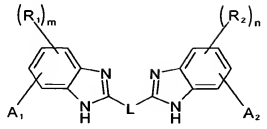


wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

33. A compound of Formula (II):



(II)

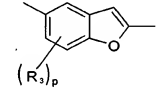
wherein:

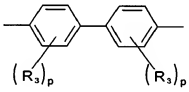
m is an integer from 0 to 3;

n is an integer from 0 to 3;

R_1 and R_2 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

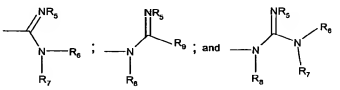
L is selected from the group consisting of:





wherein each p is independently an integer from 0 to 4 and each R_3 is independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

A_1 and A_2 are each independently selected from the group consisting of:

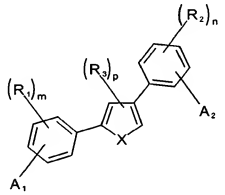


wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

34. A compound of Formula (III):



(III)

wherein:

m is an integer from 0 to 4;

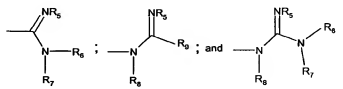
n is an integer from 0 to 4;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:

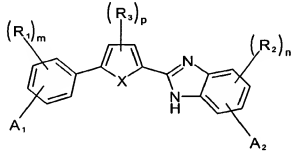


wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

35. A compound of Formula (IV):



(IV)

wherein:

m is an integer from 0 to 4;

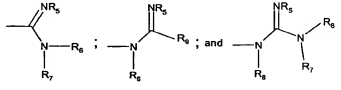
n is an integer from 0 to 3;

p is an integer from 0 to 2;

R_1, R_2, and R_3 are each independently selected from the group consisting of alkyl, halo, hydroxyl, alkoxyl, aryloxyl, and aralkyloxyl;

X is selected from the group consisting of O, S, and NR_4, wherein R_4 is H, alkyl, or alkoxyl;

A_1 and A_2 are each independently selected from the group consisting of:



wherein:

R_5, R_6, R_7, R_8, and R_9 are each independently selected from the group consisting of H, alkyl, cycloalkyl, aryl, aralkyl, hydroxyl, alkoxyl, hydroxyalkyl, hydroxycycloalkyl, alkoxycycloalkyl, aminoalkyl, acyloxyl, alkylaminoalkyl, and alkoxycarbonyl; or

R_5 and R_6 together represent a C_2 to C_{10} alkyl, C_2 to C_{10} hydroxyalkyl, or C_2 to C_{10} alkylene.

36. A pharmaceutical formulation comprising:

(a) a compound of Formula (I-IV); and

(b) a pharmaceutically acceptable carrier.

37. A method of preparing a compound of Formula (II), the method comprising refluxing a mixture of one of:

(a) 4,4'-diformyl-1,1'-biphenyl, 4-amidino-1,2-phenylenediamine hydrochloride hemihydrate, and 1,4-benzoquinone in a protic solvent to form a reaction product; and

(b) benzo[b]furan-2,5-dicarboxaldehyde, 4-*N*-isopropylamidino-1,2-phenylenediamine hydrochloride hemihydrate, and 1,4-benzoquinone in a protic solvent to form a reaction product.

38. The method of Claim 37, the method further comprising:

(a) dissolving the reaction product of one of step (a) and step (b) in a protic solvent to form a reaction mixture; and

(b) treating the reaction mixture with a solvent saturated with HCl to form a hydrochloride salt.

Abstract of the Disclosure

Novel dicationic compounds for *in vitro* activity against metronidazole-sensitive and -resistant *T. vaginalis* isolates. Six compounds in three different structural classes demonstrated IC_{50} concentrations that were not elevated in the metronidazole resistant isolate, suggesting that their activity is not affected by parasite mechanisms that confer resistance to 5-nitroimidizoles. The most effective compound gave IC_{50} values of 1μMol or less against both metronidazole resistant and sensitive isolates.

Evaluation of Dicationic Compounds for Activity against *Trichomonas vaginalis*

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Running title: Dicationic Compound Activity against *T. vaginalis*

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Abstract

Treatment of *Trichomonas vaginalis* infections with 5-nitroimidizole compounds such as metronidazole or tinidazole is usually effective. However, the not uncommon occurrence of clinical isolates resistant to 5-nitroimidiazole drugs, combined with patients that have hypersensitivity reactions to these compounds, suggests a need for additional therapeutic agents, preferably ones that can be administered orally. To this end, we have tested 44 novel cationic compounds for in vitro activity against metronidazole-sensitive and -resistant *T. vaginalis* isolates. Six compounds in three different structural classes demonstrated promising IC_{50} concentrations that were not elevated in the metronidazole resistant isolate, suggesting that their activity is not affected by parasite mechanisms that confer resistance to 5-nitroimidizoles. Interestingly, efficacy of the novel compounds was also largely unaffected by whether the assays were performed under aerobic or anaerobic conditions. The most effective compound gave IC_{50} values of 1μMol or less against both metronidazole resistant and sensitive isolates. As some of the compounds that are effective against *T. vaginalis* also show activity against other protozoan parasites, their potential for further clinical development is enhanced.

Trichomoniasis is a common sexually transmitted disease caused by the protozoan parasite *Trichomonas vaginalis*. An estimated 170 million persons are infected with *T. vaginalis* worldwide (24). Clinical manifestations range from an asymptomatic presentation to vaginitis, dyspareunia, and strawberry cervix in women and urethritis in men. In addition to these direct symptoms, trichomoniasis has also been associated with premature birth, low infant birth weight, and increased susceptibility to HIV infection (7, 20).

Metronidazole has been the principal drug prescribed for treatment of trichomoniasis infections since it was introduced in 1960 (12). Although resistance to metronidazole was first reported in 1962 (17), it is still effective, successfully treating approximately 90-95% of infections (5). However, metronidazole treatment does not cure all patients and recognition of resistance is increasing. Requests to the CDC for evaluation of metronidazole resistance in clinical isolates have increased from 15 in 1995 to over 100 in 2003 (Secor et al., unpublished observations). In addition, side effects such as gastrointestinal discomfort and nausea are commonly reported (18) and, along with hypersensitivity reactions (15), can be severe enough to preclude metronidazole use for treating some individuals.

We recently reported that tinidazole is efficacious against *T. vaginalis* isolates at lower minimal lethal concentrations (MLCs) than metronidazole (8), a finding also supported by clinical observations (19). Like metronidazole, tinidazole is a 5-nitroimidazole and isolates with very high levels of resistance to metronidazole also have increased tinidazole MLCs (8). In addition, although tinidazole use results in fewer common side effects than metronidazole, it is possible that persons with hypersensitivity

reactions to metronidazole may also have adverse reactions to tinidazole. Taken together, although tinidazole may prove to be useful in many cases of metronidazole treatment failure, identification of non-nitroimidazole compounds that have efficacy against trichomonads is desirable.

Dicationic, aromatic diamidine compounds that bind the minor groove of DNA have antimicrobial activity against a wide spectrum of protozoan parasites (23). For example, pentamidine is used to treat African trypanosomiasis and antimony-resistant leishmaniasis. However, pentamidine must be administered parenterally, it causes potentially severe host side effects, and drug resistance among parasites is emerging. These factors have led to recent research on compounds that are structurally related to pentamidine and retain anti-parasite activity but demonstrate decreased toxicity for mammalian cells. Chemically synthesized diamidine compounds that preferentially bind the minor groove of DNA have activity against *Cryptosporidium parvum* (2), *Leishmania donovani* (1,22), *Plasmodium falciparum* (1), *Trypanosoma brucei* (14), *T. cruzi* (22) and the fungi *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* (10,11). In addition, appropriate chemical design of prodrugs for these compounds can confer systemic bioavailability following oral administration (23). Such broad-spectrum activity for these related drugs stimulated us to evaluate them for activity against *T. vaginalis*.

Materials and Methods

Parasite Isolates and Compounds

CDC reference strains 085 and 520 that are metronidazole resistant and sensitive, respectively, were maintained at 37°C in Diamond's trypticase-yeast-maltose medium

(TYM; pH 6.0). Metronidazole, tinidazole, pentamidine, and berenil were purchased from Sigma Chemical Co. (St. Louis, MO). DB75 (9), DB289, (3), DB181 and 249 (4), DB690 (13), DB673, (21) and DB818 (Lee, et al., manuscript in preparation) were synthesized as previously reported; purity was determined by NMR and TLC. The syntheses of DB507 and DB364 are outlined below.

4,4'-Bis{2-[(4-amidino) benzimidazolyl]}biphenyl tetrahydrochloride (DB507). A mixture of 4,4'-diformyl-1,1'-biphenyl (0.21 g, 0.001 mole), 4-amidino-1, 2-phenylenediamine hydrochloride hemihydrate (0.39 g, 0.002 mole) and 1,4-benzoquinone (0.216 g, 0.002 mole) in ethanol was heated at reflux for 12 h. The solvent was reduced to one third, followed by dilution with ether and then filtration, yielding a dark solid. The solid was dissolved in large volume of hot ethanol and filtered; the solution was treated with 10 ml HCl gas saturated ethanol and stirred. The solvent was reduced to one third and diluted with ether. A dark hydrochloride salt precipitated, which was filtered, washed with ether, and dried in vacuum at 75 ^{0}C for 24 h to yield 0.43 g (66%); mp >300 ^{0}C dec.; ^{1}H-NMR (DMSO-d_6): 8.35(d, 4H, J = 7.6 Hz), 8.21(s, 2H), 8.02(d, 4H, J = 7.6 Hz), 7.85(d, 4H, J = 8.4 Hz), 7.50(d, 4H, J = 8.4 Hz); ^{13}C NMR (DMSO-d6): 166.0, 153.2, 141.4, 137.5, 128.4, 127.8, 126.9, 123.4, 122.6, 116.2, 115.1; FAB MS: m/e 483(M^++1); Analysis calculated for $C_{29}H_{22}N_8.4HCl.1.5H_2O$: C, 53.41; H, 4.46: N, 17.09. Found: C, 52.97; H, 4.61; N, 17.17.

2,5-Bis{2-[5-(*N*-isopropylamidino)benzimidazoyl]}benzo[b]furan tetrahydrochloride (DB364). A protocol similar to that described above for DB507, involving the condensation of benzo[b]furan-2,5-dicarboxaldehyde and 4-*N*-isopropylamidino-1,2-phenylenediamine gave a metallic green solid in 69% yield; mp

285 ^{0}C-290 ^{0}C. ^{1}H NMR (DMSO-d_6/80°C) 8.71 (s, 1H), 8.36 (d, 1H, J = 8.8 Hz) 8.08(d, 2H, J = 9.2 Hz), 7.98 (d, 1H, J = 8.8 Hz), 7.96 (s, 1H), 7.85 (d, 1H, J = 8.8 Hz), 7.82 (d, 1H, J = 8.8 Hz), 7.64 (d, 1H, J = 8.8 Hz), 7.61 (d, 1H, J = 8.8 Hz), 4.02 (broad q, 2H, J = 6 Hz), 1.32(broad d, 12H, J = 6 Hz). ^{13}C NMR (DMSO-d_6/D_2O/80°C) 162.9, 162.6, 157.5, 152.9, 147.4, 145.3, 141.1, 138.7, 137.8, 134.9, 129.2, 126.9, 125.7, 125.0, 124.5, 123.8, 123.2, 121.4, 116.9, 116.1, 115.7, 115.5, 113.9, 109.4, 46.2, 46.1, 21.6(signals overlap). Analysis calculated for $C_{30}H_{30}N_80$ 4HCl 0.5H_2O: C, 53.49; H, 5.23; N, 16.64. Found: C, 53.53; H, 5.29; N, 16.45.

Assays

Compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma) and further diluted with Diamond's TYM media to reach assay concentrations. Two types of assays were performed on the synthesized cationic compounds being evaluated. An initial screen was performed using the standard MLC assay (8, 16), with a maximum concentration of 20μM for 44 test compounds. After 48 hours of incubation at 37°C, plates were examined using an inverted phase-contrast microscope. The lowest drug concentration at which no motile trichomonads were observed was recorded as the MLC. Each compound was tested at least twice under both aerobic and anaerobic conditions. Anaerobic conditions were generated using a GasPak jar and CO_2-generating GasPak Plus anaerobic system envelopes (Becton Dickinson, Sparks, MD) and monitored with GasPak disposable anaerobic indicator strips (Becton Dickinson). Compounds that showed no activity were not tested further.

The structures of the 6 compounds that had some effect in the MLC assay as well as 3 related but ineffective compounds are shown in Figure 1. To further evaluate their activity, a second type of assay was used to determine the concentration at which 50% of the parasite growth was inhibited (IC_{50}). In these assays, 0.5 μCi of tritiated thymidine (Perkin-Elmer, Boston, MA) was added to each well of a standard assay at the initiation of culture. At 48hr of incubation under either aerobic or anaerobic conditions, cells were harvested onto glass fiber filters (Wallac, Turku, Finland) using a Tomtec cell harvester (Hamden, CT). Incorporated thymidine was detected using Betaplate (Wallac) scintillation fluid and plate reader. The resulting counts per minute over the compound's concentration range were utilized to calculate IC_{50}s using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Results and Discussion

Out of 44 dicationic compounds tested in the MLC assay, 6 showed sufficient activity for further evaluation and calculation of their IC_{50}. The structures of these compounds and three related compounds are shown in Figure 1. Table 1 contains their in vitro IC_{50} activities against metronidazole-sensitive and -resistant *T. vaginalis* isolates as well as comparison data for the nitroimidazoles metronidazole and tinidazole. We have also included the evaluation of the classical antiprotozoan dicationic molecules pentamidine and berenil. Interestingly, both of these latter two compounds are not effective against *T. vaginalis*. In contrast, furamidine (DB75), the parent molecule in the 2,5-diphenylfuran family of diamidines, shows good activity that is comparable to that of metronidazole against 520, the metronidazole sensitive isolate. It is clearly more effective

than either metronidazole or tinidazole against the resistant strain 085 under aerobic conditions. These data suggest, as expected based on structure, a different mode of action for DB75 as compared to the nitroimidazoles. The *N*-alkyl analogs of furamidine, DB181 and DB249, show similar in vitro effectiveness. Interestingly, DB673, a guanidino analog of DB75, and DB690, the 2,4-diphenylfuran isomer of DB75, are not effective against *T. vaginalis* in these assays. Thus, we find that the 2,5-diphenyl furan family of dications is quite effective versus *T. vaginalis* in vitro; but the activity is quite sensitive to structure as demonstrated by the lack of activity of DB690 and DB673. Not unexpectedly, DB289, the prodrug for DB75, was generally ineffective in vitro as the mammalian biochemical pathways necessary to convert it to the active form were not present. However, DB289 can be administered orally to provide systemic, efficacious concentrations of DB75 and is currently in phase II trials for treatment of African trypansomiasis, having successfully completed phase I clinical trials (23). If continued progress on the clinical path result in licensing of this compound, it should be evaluated for clinical effectiveness against trichomonads, especially those that are resistant to metronidazole and tinidazole.

The bis-benzimidazoles DB364 and DB507 are also quite effective anti-trichomonads. Interestingly, DB507 has activity against the metronidazole-resistant 085 strain but not against the metronidazole-sensitive 520 isolate. Thus, DB507 may be a useful tool to evaluate the biochemical basis of *T. vaginalis* resistance to metronidazole. The most effective dication studied is the mono-benzimidazole DB818. This compound demonstrated IC_{50} values of 1 μMol or less for both metronidazole sensitive and resistant isolates under either aerobic or anaerobic conditions. The mono-benzimidazole DB818

has been successfully used in vivo to treat a different protozoal infection in an experimental model without overt evidence of toxicity to the host (Brun and Boykin, unpublished observations). Clearly, like the 2,5-diphenylfurans, this compound merits further evaluation in vivo versus *T. vaginalis*.

In conclusion, aromatic diamidines, which bind to the minor groove of DNA at AT sites, have potential as anti-trichomonad agents. A recent paper (6) reports that another class of dicationic DNA minor groove binding compounds (bis-quarternary quinoliniums) is also effective against *T. vaginalis*. Together, these studies reinforce the utility of compounds that bind the minor groove of DNA as effective anti-trichomonad agents and substantiate the need for further research on this interaction.

Acknowledgements. A. L. C. was supported by an appointment to the Emerging Infectious Diseases Fellowship Program administered by the Association of Public Health Laboratories (APHL). The work was funded by the Office of Minority and Women's Health, National Center for Infectious Diseases, Centers for Disease Control and Prevention. The compound synthesis activities were supported by NIH grant No. NAID RO1AI46365. In addition, this work was performed with support in conjunction with the VAMC Atlanta and the Atlanta Research and Education Foundation

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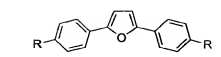
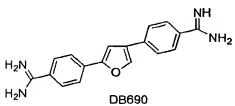
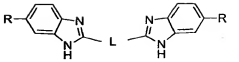


DB75 R = -C=NH(NH_2)
DB289 R = -C=NOMe(NH_2)
DB181 R = -C=N-*i*-Pr(NH_2).
DB249 R = -C=NH-*c* -cyclohexyl(NH_2)
DB673 R = -NH(C=NH)(NH_2)



DB690



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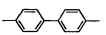
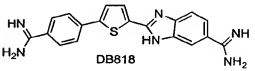
DB507 L =  R = -C=NH(NH2)



DB818

Figure 1. Structures of dicationic compounds evaluated in this study.

Table 1. IC_{50} values ($\mu M \pm SEM$) of compounds evaluated in this study.

	085 aerobic		085 anaerobic		520 aerobic		520 anaerobic	
Compound	n		n		n		n	
Metronidazole	61	302.6 ± 22.2	62	12.3 ± 23.8	38	18.2 ± 4.25	31	1.89 ± 0.77
Tinidazole	5	45.1 ± 5.38	4	4.81 ± 1.17	2	1.48 ± 0.12	2	0.004 ± 0.0
Pentamidine	3	no effect	3	no effect	3	no effect	3	no effect
Berenil	3	no effect	3	no effect	3	no effect	3	no effect
DB75	8	8.12 ± 2.45	7	18.6 ± 19.8	4	18.6 ± 6.43	4	57.9 ± 19.6
DB289	3	39.1 ± 25.1	2	no effect	2	no effect	2	no effect
DB181	7	6.44 ± 3.89	7	9.41 ± 7.92	7	6.60 ± 1.76	7	3.91 ± 1.06
DB249	6	22.4 ± 13.7	6	10.2 ± 8.47	2	15.9 ± 0.74	3	13.9 ± 3.74
DB364	6	7.27 ± 3.76	5	37.6 ± 43.9	2	44.7 ± 9.33	3	143.1 ± 127
DB507	8	7.79 ± 4.20	6	25.3 ± 43.1	2	no effect	2	no effect
DB690	7	no effect	6	no effect	3	no effect	3	no effect
DB673	7	no effect	6	no effect	3	no effect	3	no effect
DB818	7	0.27 ± 0.04	7	0.50 ± 0.42	6	0.98 ± 0.19	6	1.24 ± 0.39